

RESEARCH ARTICLE

Is the spider a good biological control agent for *Plutella xylostella* (Lepidoptera: Plutellidae)?

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ABSTRACT. Spiders, as predators of insects and other invertebrates, are an important part of the natural enemies, and they are recognized as an important biological control agent. *Plutella xylostella* (Linnaeus, 1758), the diamondback moth (DBM), is a well-known and destructive insect pest of brassicaceous crops worldwide. Here, we analyzed the functional responses of four spiders (Araneae) – *Ebrechtella tricuspidata* (Fabricius, 1775) (Thomisidae), *Pardosa laura* (Karsch, 1879) (Lycosidae), *Pardosa astrigera* (Koch, 1878) (Lycosidae), and *Pardosa pseudoannulata* (Bösenberg & Strand, 1906) (Lycosidae) – on *P. xylostella* larvae. We also analyzed intraspecific disturbances in the predation reaction and the intensity of scrambling competition of the spiders to *P. xylostella* larvae. Our results demonstrated that the functional responses of four spiders of different genera were in line with the Holling II model. Two Lycosidae spiders (*P. astrigera* and *P. pseudoannulata*) had the potential to control *P. xylostella*, and female and male spiders that belonged to the same species had different functional responses to *P. xylostella*. The functional responses of female predation of *P. astrigera*, *P. laura*, and *P. pseudoannulata* was stronger than the males, but male *E. tricuspidatus* had stronger functional responses to predation than females. We used the Hassell model to describe the intraspecific disturbance experiments of four spiders. There were intraspecific disturbances in the predation reactions of spiders, indicating that the predation ratio of spiders decreased in relation to the increase of its density, and with the increase of spider density, the intensity of scrambling competition of the spider increased.

KEY WORDS. Diamondback moth, *Ebrechtella tricuspidata*, *Pardosa laura*, *Pardosa astrigera*, *Pardosa pseudoannulata*, predation.

INTRODUCTION

Plutella xylostella (Linnaeus, 1758) is a well-known and destructive insect pest of brassicaceous crops worldwide (Talekar and Shelton 1993). The annual cost around the world for *P. xylostella* pest control measures has been routinely quoted at US\$ 1 billion from 1993 to 2012 (Zalucki et al. 2012). *Plutella xylostella* is very difficult to control for several reasons, including diversity and abundance of host plants, the lack or disruption of its natural enemies, high reproductive potential (over 20 generations per year in the tropics), and its genetic elasticity, which facilitates rapid development of resistance to insecticides (Sarfranz et al. 2006).

In the locations where brassicaceous crops are planted, large amounts of pesticides have been used to control pest. Under such high selection pressures, pests often develop high levels of

insecticide resistance (Shelton et al. 1993). Unfortunately, the most prominent one is *P. xylostella*, which is naturally difficult to control (Mota-Sanchez et al. 2002, Sarfranz and Keddie 2005). Zhao et al. (2002) reported that most populations of *P. xylostella* are susceptible to an insecticide called Spinosad, but control failures occurred in several locations of Hawaii. Sarfranz and Keddie (2005) reported significant resistance to almost every synthetic insecticide applied in the field of many crucifer-producing regions. For these reasons, we urgently need more environmentally friendly methods, such as biological methods, to control pests and to alleviate the current situation (Shelton et al. 1993).

Spiders are an important part of the natural enemies, and they are recognized as an important biological control agent (Matteson 2000, Turnbull 1973). Rodrigues et al. (2013) thought a balance could be reached between the number of spiders and pests that was equivalent to a natural-control process.

For example, Nyffeler et al. (1987) reported that the Lycosidae spiders can be predators of the pests as *Heliothis zea* (Boddie, 1850) (Lepidoptera: Noctuidae) and *Alabama argillacea* (Hübner, 1823) (Lepidoptera: Noctuidae) in cotton fields. In addition, Pérez-Guerrero et al. (2014) confirmed the considerable potential and value of some cursorial spiders in the biological control of lepidopteran cotton pests.

In the present study, we researched three Lycosidae spiders and one Thomisidae spider that have been used for the biological control of some key lepidopteran pests (Senior et al. 2016). In an effort to study these biological control agents, we defined several objectives. First, we compared the differences between the functional responses of the four species of spiders, and we investigated the effect of spider gender on the functional responses toward *P. xylostella* larvae. Second, we analyzed intraspecific disturbances and the intensity of scrambling competition of these four spiders species. Finally, we systematically evaluated the predation of the four species of spiders toward *P. xylostella*.

MATERIAL AND METHODS

Plutella xylostella and spiders rearing procedure

Ebrechtella tricuspidata (Fabricius, 1775) (Thomisidae), *Pardosa laura* (Karsch, 1879) (Lycosidae), *Pardosa astrigera* (Koch, 1878) (Lycosidae), and *Pardosa pseudoannulata* (Bösenberg & Strand, 1906) (Lycosidae) were chosen for this study because they are preponderant species that are common and abundant in tea gardens (Cui et al. 2012). The spiders were collected from the fields of Huazhong Agricultural University in Wuhan (30°52'N; 114°31'E), Hubei Province, China, between May and October 2015. They were kept individually in cylindrical glass tube (2.5 cm diameter × 10.0 cm high) with a layer of sponge (1.5–2.0 cm thick) moistened with water on the bottom. The tubes were plugged with cotton. The spiders were kept in the chambers under the following laboratory conditions: 26 ± 0.5 °C, relative humidity of 60% ± 3%, and under a light: dark cycle of 14 h:10 h (lights turned on at 8:00 am). Every two days, the spiders were fed adult *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae).

Plutella xylostella were obtained from the Vegetable Research Institution of Wuhan, Hubei Province, China, where they were placed together in a cage and fed with cabbage. They were kept under the same laboratory conditions as the spiders and their cages were cleaned every five to six days.

Functional response of spiders to third instar larvae of *P. xylostella*

At the beginning of the experiment, the spiders were starved for 48 hours to eliminate different degrees of hunger. Three days after the last molt, one adult spider of each species was placed in separate containers. Each container contained one spider and third instar larvae of *P. xylostella* at different densities (10, 15, 20, 25, and 30); there were also control containers that

contained no *P. xylostella*. The experiments were repeated with at least three replicates per density level. The number of prey that survived after 24 hours was recorded. The functional responses of each gender of spider in each family were analyzed according to Holling II model (Holling 1959).

Intraspecific disturbance of the predation reaction and intensity of scrambling competition of the spiders toward *P. xylostella* larvae

Three days after the last molt, adult spiders (n = 15) from each species were placed into six groups with 30 third instar larvae of *P. xylostella* in each group: control group (no spiders), and 1, 2, 3, 4, and 5 spiders per container. Spiders were starved for 48 hours before the tests to standardize the hunger level. Each density experiment was repeated at least three times. The number of surviving *P. xylostella* in each container after 24 hours was recorded. The intraspecific disturbance of the spiders predation reaction on *P. xylostella* larvae were calculated according to the Hassell model (Hassell and Varley 1969).

Data analyses

All functional-response equations were fitted using a Holling II model, as shown in the following equation:

$$N_a = \frac{a' \times T \times N}{1 + a' \times T_h \times N} \quad (1)$$

Where, N_a was the number of the third instar larvae of *P. xylostella* predated by the spider, N was the density of the third instar larvae of *P. xylostella*, a' was the attack coefficient of the spider, and T_h was the handling time of the spider per one *P. xylostella*. T was equal to one because the functional response experiment lasted for one day. Equation (1) is a nonlinear-curve equation, so we took the inverse of the equation to produce a linear equation:

$$\frac{1}{N_a} = \frac{1}{a'} \times \frac{1}{N} + T_h \quad (2)$$

N_a and N were recorded based on the experimental result; a' and T_h were calculated using Equation (2).

All experimental data of intraspecific disturbance of the predation reaction of spiders on *P. xylostella* larvae were fitted using the Hassell model:

$$E = QP^{-m} \quad (3)$$

Where, E was the predation ratio of the spider, P was the spider density, Q was the searching constant, and m was the interference constant. Q and m were the parameters that were estimated according to the equation:

$$E = \frac{N_a}{N \times P} \quad (4)$$

Here, N_a was the total number of *P. xylostella* preyed on by all of the spiders, N was the *P. xylostella* density, and P was the spider density.

The predation competition of spiders on *P. xylostella* was equal to the scrambling competition, so the intensity of scrambling competition (I) of spiders was determined using by the following equation:

$$I = \frac{E_1 - E_p}{E_1} \quad (5)$$

Where, E_1 was the predation ratio of one spider and E_p was the predation ration with P density of spiders.

The relationship between the intensity of scrambling competition (I) and the density of spiders (P) was analyzed using the following equation:

$$I = a \lg P + b \quad (6)$$

a and b were the parameters that were estimated according to the values of I .

All collected data were analyzed using MS Excel 2007.

RESULTS

Functional response of spiders to *P. xylostella*

The functional-response equations of each gender for the four spiders species are shown in Table 1. The fitting coefficients of the eight equations ranged from 0.9031 to 0.9672, indicating these eight equations fit well. Compared to *E. tricuspidata* and *P. laura*, *P. astrigena* and *P. pseudoannulata* had stronger predation reactions toward the third instar larvae of *P. xylostella*. Among the four types of spiders, *P. pseudoannulata* was the greatest natural enemy to *P. xylostella*, and *P. laura* was the weakest predator.

The functional-response equations of female and male *P. pseudoannulata* to the third instar larvae of *P. xylostella* were $1/N_a = 0.8913/N_t + 0.0382$ and $1/N_a = 0.9736/N_t + 0.0411$, respectively. The maximum number of third instar larvae of *P. xylostella* predated by both genders of *P. pseudoannulata* (26.2 larvae of female and 24.3 larvae of male) were significantly higher than those predated by any gender of the other three spiders. At the same time, the highest a' (that is, 1.1219 h^{-1}) and the shortest T_h (that is, 0.382 h) were reached by female *P. pseudoannulata* (Table 1).

At the same time, the results showed that female and male spiders that belonged to the same species had different functional responses to *P. xylostella*. Three of the four types of female spiders (*P. laura*, *P. astrigena*, and *P. pseudoannulata*) had stronger functional responses than the males of the same species. The functional-response equation of female and male *E. tricuspidata* in response to the third instar larvae of *P. xylostella* were $1/N_a = 1.2555/N_t + 0.0801$ and $1/N_a = 1.8392/N_t + 0.0652$, respectively. The maximum number (N_{\max}) of third instar larvae of *P. xylostella* predated by male *E. tricuspidata* was 15.3, which

Table 1. Functional responses of spiders to third instar larvae of *Plutella xylostella*. (a') the attack coefficient of the spider, (N) the density of the third instar larvae of *P. xylostella*; (N_a) the number of the third instar larvae of *P. xylostella* predated by the spider; (r) the fitting coefficient; (T_h) the handling time of spider per one *P. xylostella*; (N_{\max}) the maximum number of third instar larvae of *P. xylostella* predated by the spider.

Spider	Gender	Equation of functional response	r	T_h (h)	a (h^{-1})	N_{\max} (larvae)
<i>Ebrechtella tricuspidata</i>	Female	$1/N_a = 1.2555/N + 0.0801$	0.9297	0.0801	0.7965	12.5
	Male	$1/N_a = 1.8392/N + 0.0652$	0.9415	0.0652	0.5437	15.3
<i>Pardosa laura</i>	Female	$1/N_a = 1.2234/N + 0.0792$	0.9113	0.0792	0.8174	12.6
	Male	$1/N_a = 1.3868/N + 0.0838$	0.9031	0.0838	0.7211	11.9
<i>Pardosa astrigera</i>	Female	$1/N_a = 1.1788/N + 0.0479$	0.9561	0.0479	0.8483	20.9
	Male	$1/N_a = 1.0810/N + 0.0503$	0.9305	0.0503	0.9251	19.9
<i>Pardosa pseudoannulata</i>	Female	$1/N_a = 0.8913/N + 0.0382$	0.9672	0.0382	1.1219	26.2
	Male	$1/N_a = 0.9736/N + 0.0411$	0.9316	0.0411	1.0271	24.3

was significantly higher than the female (12.5). The a' of the male *E. tricuspidata* (0.7965/h) was also higher than the female (0.5437/h) (Table 1).

Intraspecific disturbance and intensity of scrambling competition of the spider predation on *P. xylostella* larvae

According to N_a (Table 2) and Equation (4), we calculated the values of E_p shown in Table 2. With the increase of spider density, N_a and E_p of each spider decreased gradually. The searching constant, Q , and the interference constant, m , of the Hassell model were calculated according to E_p and the spider density, as shown in Table 3. The fitting coefficients of the four equations ranged from 0.9424 to 0.9863, indicating that the Hassell model can be used to describe the intraspecific disturbance situation of the spider. There were intraspecific disturbances in the predation reaction of spiders.

Using the Equation (5), we calculated the value for I , as shown in Table 2. At the same time, we calculated the values of a and b parameters of Equation (6), as shown in Table 4. The relationship between the intensity of scrambling competition, I , and spider density, P , was positively correlated, indicating that the intensity of the scrambling competition increased with the increasing spider density.

DISCUSSION

Plutella xylostella is one of the major pests of Chinese cruciferous plants, and they have caused a great deal of harm to Chinese vegetable crops for many years (Lin et al. 2006). Currently, under high pesticide selection pressures, *P. xylostella* have developed high levels of insecticide resistance in some areas. This means that simple chemical methods are not sufficient or efficient. New biological control strategies

Table 2. Predation ratio and intensity of scrambling competition of spiders toward *Plutella xylostella*. (E_p) the predation ratio with P density of spiders, (I) the intensity of the scrambling competition, (N_a) the total number of *P. xylostella* preyed on by all of the spiders in the container.

Spider	Spider density	N_a	E_p	I
<i>Ebrechtella tricuspidata</i>	1	12.3	0.4111	0.0000
	2	8.3	0.1383	0.6636
	3	6.0	0.0667	0.8378
	4	4.7	0.0392	0.9046
	5	2.7	0.0180	0.9562
<i>Pardosa laura</i>	1	13.3	0.4433	0.0000
	2	10.7	0.1783	0.5978
	3	7.7	0.0856	0.8069
	4	5.0	0.0417	0.9059
	5	2.3	0.0153	0.9655
<i>Pardosa astrigera</i>	1	20.7	0.6900	0.0000
	2	16.7	0.2783	0.5967
	3	12.0	0.1333	0.8068
	4	8.3	0.0692	0.8997
	5	6.7	0.0447	0.9352
<i>Pardosa pseudoannulata</i>	1	24.7	0.8233	0.0000
	2	18.3	0.3550	0.5688
	3	12.3	0.1700	0.7935
	4	9.3	0.0775	0.9059
	5	6.3	0.0422	0.9487

Table 3. The searching constant and interference constant of the intraspecific disturbance equations of the spiders.

Spider	Searching constant	Interference constant	Fitting coefficient
<i>Ebrechtella tricuspidata</i>	0.4590	1.867	0.9813
<i>Pardosa laura</i>	0.5631	1.981	0.9424
<i>Pardosa astrigera</i>	0.7776	1.710	0.9863
<i>Pardosa pseudoannulata</i>	1.0037	1.826	0.9646

are increasingly needed to control insect pests (Saleh et al. 2017); however, the role of natural predators of *P. xylostella* in agricultural ecosystems has not been taken seriously. Research and practice of biological control have concentrated mainly on parasitoids and diseases on this insect pest (Talekar and Shelton 1993). The spiders are the most abundant predators recorded in grain crops, and their assemblages cause high mortality on pest populations (Riechert 1999, Whitehouse and Lawrence 2001, Pearce and Zalucki 2005). Therefore, the present article has focused on four common and abundant spiders in order to explore the potential to control the diamondback moth. Understanding the relationship between the spider predator and prey has been a central goal in controlling the worldwide pest *P. xylostella*. Since 1959, Holling's prey-dependent Type II functional response, a model that is a function of prey

Table 4. Intensity of the scrambling-competition equation of each type of spider.

Spider	Intensity of scrambling-competition equation	r
<i>Ebrechtella tricuspidata</i>	$I = 1.358 \lg P + 0.107$	0.9177
<i>Pardosa laura</i>	$I = 1.386 \lg P + 0.0781$	0.9547
<i>Pardosa astrigera</i>	$I = 1.355 \lg P + 0.0837$	0.9443
<i>Pardosa pseudoannulata</i>	$I = 1.379 \lg P + 0.0693$	0.9611

(I) intensity of scrambling competition, (P) the density of the spiders, (r) fitting coefficient.

abundance, has served as the basis for a robust literature on predator-prey theory (Skalski and Gilliam 2001).

Similar to other studies that have modelled the functional response of spiders using the Holling II model, the present study found that with an increase in the *P. xylostella* density, the feeding amount of the spider first increased, but then reached a stable level. Lin et al. (2006) found that the predator function of the *Hylyphantes graminicola* (Sundevall, 1830) (Araneae: Linyphiidae) on the *P. xylostella* larvae belongs to the Holling II model, and *H. graminicola* had predation preferences for the larvae of *P. xylostella*. Zeng et al. (2016) found that the functional predation response of *P. pseudoannulata* and *Pardosa procurva* (Yu & Song, 1988) (Araneae: Lycosidae) on the fourth instar larvae of *P. xylostella* fit well with the Holling II model, and the Holling model could perfect the predation effect. The functional-response curves for these spiders seem to be a typical type-II functional response, with the prey consumed increasing to a plateau as the aphid density increases (Mansour and Heimbach 1993). A possible explanation is that the stomach capacity of the spider is limited. When the predation needs are met, it no longer needs prey.

More spider species were used for the experiments of the present study in comparison to other research reports, which have been mainly focused on the effects of temperature and space volumes in relation to the functional response of the predator. Duan and Zhang (2004) reported that the predation of *Menochilus sexmaculata* (Fabricius, 1781) (Coleoptera: Coccinellidae) on *Macrosiphoniella sanborni* (Gillette, 1908) (Homoptera: Aphididae) was affected by temperature, vessel space, and prey density.

In the present study, we concentrated on the effect of spider gender on the functional response of the spider toward *P. xylostella* because there is little research on this topic. Previous studies reported that female spiders are more ferocious, and their predation amounts are larger, such that females consumed more than males under similar conditions (Walker and Rypstra 2001, Mohsin et al. 2015). This is presumed to be due to the female requiring more energy to lay eggs, care for brood, and reproduce and hibernate in upcoming seasons. Mukhtar et al. (2012) reported female have to fulfill their energy requirements to maximize their breeding. In addition, Walker and Rypstra (2001) found that *Rabidosia rabida* (Walckenaer, 1837) (Araneae: Lycosidae) females attack more prey than males. However, in the present study, in contrast with past research, the maximum number of *P. xylostella* larvae predated and the attack coefficient of male *E. tricuspidata*

were significantly higher than female *E. tricuspidata*. On the other hand, among the four spiders species, *P. pseudoannulata* was the greatest natural enemy to *P. xylostella*. One possibility for this finding is that *P. pseudoannulata* has the largest body size. It needs more prey to meet its own energy demands.

There were intraspecific disturbance in the predation reaction of the four types of spiders. When five spiders were placed in one reaction container, we observed that they did not attack each other. However, they took more time to search for the prey, the interference between individual spiders increased, and the preying capacity of each spider decreased. The correlation between intensity of scrambling competition, I, and spider density, P, was positive. Wu and Wang (1987) also found that the predation of *P. pseudoannulata* increased with the increase in the number of prey, and decreased with the increase of its density. Predatory arthropods can reduce the impact of these invertebrates and limit the number of pests in agroecosystems. Predators displayed lower predation rates when kept together compared to the predation rates of individual spiders kept alone (Pérez-Guerrero et al. 2013).

According to the results of the present study, we found that two species of Lycosidae spiders – *P. astrigera* and *P. pseudoannulata* – had the potential to control *P. xylostella*. We also found that male *E. tricuspidata* were more suitable to be released in the planted brassicaceous fields. However, the functional response and intraspecific disturbance experiments were only carried out under laboratory conditions in which predator and prey were placed in a small container. It is difficult to extrapolate to an actual situation in the field, where the size of the activity space, the niche overlap index, the developmental period, the arthropod community, and the density of predator and prey can all affect the predator behavior. In order to fully evaluate the role of spiders as predators for the control of *P. xylostella*, future studies could include longer experiments for agroecosystems, and could use varied methods so as to cover the most appropriate release number of spiders for the control of *P. xylostella*.

The results showed that the functional responses of four spiders species were in line with the Holling II model. Two Lycosidae spider – *P. astrigera* and *P. pseudoannulata* – had the potential to control *P. xylostella*. The functional response of the female *P. astrigera*, *P. laura*, and *P. pseudoannulata* were stronger than the males; the opposite results were found for *E. tricuspidata*. Intraspecific interference of the four spiders were analyzed using the Hassell model. We found that the predation ratio of the spider decreased with the increase of predator density. At the same time, the correlation between the intensity of scrambling competition and spider density was positive, indicating that the intensity of the scrambling competition increased with an increase in spider density.

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